

- SEOUENCES <110> NATIONAL INSTITUTE FOR AGRICULTURAL RESEARCH (INRA) <120> PROCEDURE FOR THE PREPARATION OF 1,3-PROPANEDIOL STARTING FROM A RECOMBINANT MICRO-ORGANISM, IN THE ABSENCE OF COENZYME B12 OR ONE OF ITS PRECURSORS. <130> CHEP004US <140> PCT/FR00/01981 <141> 2000-07-07 <160> 10 <170> Patent IN Ver. 2.1. <210> 1 <211> 2364 <212> DNA <213> Clostridium butyricum <400> 1 atqataaqta aaggatttag tacccaaaca gaaagaataa atattttaaa ggctcaaata 60 ttaaatgcta aaccatgtgt tgaatcagaa agagcaatat taataacaga atcatttaaa 120 caaacaqaaq qccaqccaqc aattttaaqa aqaqcattqq cattqaaaca catacttgaa 180 aatatcccta taacaattag agatcaagaa cttatagtgg gaagtttaac taaagaacca 240 aggtetteae aagtatttee tgagttttet aataagtggt tacaagatga attggataga 300 ttaaataaga gaactggaga tgcattccaa atttcagaag aaagtaaaga aaaattaaaa 360 gatqtctttq aqtattqqaa tqgaaaqaca acaagtqagt tagcaacttc atatatgaca 420 gaggaaacaa gagaggcagt aaattgtgaa gtatttactg taggaaacta ctattataat 480 ggcgtaggac atgtatctgt agattatgga aaagtattaa gggttggatt taatgggatt 540 ataaatgagg ctaaggaaca attagaaaaa aacaggagta tagatcctga ttttataaag 600 aaagaaaaat tootaaatag tgttattato toatgogaag otgoaataac atatgtaaat 660 aqatatqcta aaaaqqctaa agaqattgca gataatacaa gtgatgcaaa aagaaaagct 720 gaattaaatg aaataqcaaa aatttgttca aaagtttcag gagagggagc taaatctttc 780 tatgaagcat gicaattatt tiggittatt catgcaataa taaatataga atctaatgga 840 cattctattt ctccagctag atttgatcaa tacatgtatc catattatga aaatgataaa 900 aatataacag ataagtttgc tcaagaatta atagattgta tctggattaa attaaatgat 950
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<211> 915

<212> ADN

<213> Clostridium butyricum

WO 01/04324

WO 01/04324 PCT/FR00/01981

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<211> 28

<212> ADN

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<400> 3

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28

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<211> 1158

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<213> Clostridium butyricum

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<210> 5

<211> 4963

<212> ADN

<213> Clostridium butyricum

<400> 5

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aaggotcaaa	tattaaatgo	taaaccatgt	gttgaatcag	aaagagcaat	attaataaca	420
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		agttaataaa				
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<211> 787

<212> PRT

<213> Clostridium butyrıcum

<400> 6

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Lys Ala Gln Ile Leu Asn Ala Lys Pro Cys Val Glu Ser Glu Arg Ala 20 25 30

Ile Leu Ile Thr Glu Ser Phe Lys Gln Thr Glu Gly Gln Pro Ala Ile 35 40 45

Leu Arg Arg Ala Leu Ala Leu Lys His Ile Leu Glu Asn Ile Pro Ile 50 55 60

Thr Ile Arg Asp Gln Glu Leu Ile Val Gly Ser Leu Thr Lys Glu Pro
65 70 75 80

Arg Ser Ser Gln Val Phe Pro Glu Phe Ser Ash Lys Trp Leu Gln Asp 85 90 95

Glu Leu Asp Arg Leu Asn Lys Arg Thr Gly Asp Ala Phe Gln Ile Ser 100 :05 110 Glu Glu Ser Lys Glu Lys Leu Lys Asp Val Phe Glu Tyr Trp Asn Gly Lys Thr Thr Ser Glu Leu Ala Thr Ser Tyr Met Thr Glu Glu Thr Arg Glu Ala Val Asn Cys Glu Val Phe Thr Val Gly Asn Tyr Tyr Tyr Asn Gly Val Gly His Val Ser Val Asp Tyr Gly Lys Val Leu Arg Val Gly Phe Asn Gly Ile Ile Asn Glu Ala Lys Glu Gln Leu Glu Lys Asn Arg Ser Ile Asp Pro Asp Phe Ile Lys Lys Glu Lys Phe Leu Asn Ser Val Ile Ile Ser Cys Glu Ala Ala Ile Thr Tyr Val Asn Arg Tyr Ala Lys Lys Ala Lys Glu Ile Ala Asp Asn Thr Ser Asp Ala Lys Arg Lys Ala 230 235 Glu Leu Asn Glu Ile Ala Lys Ile Cys Ser Lys Val Ser Gly Glu Gly Ala Lys Ser Phe Tyr Glu Ala Cys Gln Leu Phe Trp Phe Ile His Ala Ile Ile Asn Ile Glu Ser Asn Gly His Ser Ile Ser Fro Ala Arg Phe

Asp Gln Tyr Met Tyr Pro Tyr Tyr Glu Asn Asp Lys Asn Ile Thr Asp 300 290 295 Lys Phe Ala Gln Glu Leu Ile Asp Cys Ile Trp Ile Lys Leu Asn Asp 315 310 305 Ile Asn Lys Val Arg Asp Glu Ile Ser Thr Lys His Phe Gly Gly Tyr 330 335 325 Pro Met Tyr Gln Lys Leu Ile Val Gly Gly Gln Asn Ser Glu Gly Lys 345 Asp Ala Thr Asn Lys Val Ser Tyr Met Ala Leu Glu Ala Ala Val His 360 . 355 Val Lys Leu Pro Gln Pro Ser Leu Sor Val Arg Ile Trp Asn Lys Thr 375 370 Pro Asp Glu Phe Leu Leu Arg Ala Ala Glu Leu Tar Arg Glu Gly Leu 395 385 390 Gly Leu Pro Ala Tyr Tyr Asn Asp Glu Val Ile Ile Pro Ala Leu Val 410 415 435 Ser Arg Gly Leu Thr Leu Glu Asp Ala Arg Asp Tyr Gly Ile Ile Gly 425 420 Cys Val Glu Pro Gln Lys Pro Gly Lys Thr Glu Gly Trp His Asp Ser 440 435 Ala Phe Phe Asn Leu Ala Arg Ile Val Glu Leu Thr Ile Asn Ser Gly 455 460 450

Phe Asp Lys Asn Lys Gln Ile Gly Pro Lys Thr Gln Asn Phe Glu Glu 47C Met Lys Ser Phe Asp Glu Phe Met Lys Ala Tyr Lys Ala Gln Met Glu Tyr Phe Val Lys His Met Cys Cys Ala Asp Ash Cys Ile Asp Ile Ala His Ala Glu Arg Ala Pro Leu Pro Phe Leu Ser Ser Met Val Asp Asn Cys Ile Gly Lys Gly Lys Ser Leu Gln Asp Gly Gly Ala Glu Tyr Asn Phe Ser Gly Pro Gln Gly Val Gly Val Ala Asn Ile Gly Asp Ser Leu Val Ala Val Lys Lys Ile Val Phe Asp Glu Asn Lys Ile Thr Pro Ser 5 Glu Leu Lys Lys Thr Leu Asn Asn Asp Phe Lys Asn Ser Glu Glu Ile Gln Ala Leu Leu Lys Asn Ala Pro Lys Phe Gly Asn Asp Ile Asp Glu Val Asp Asn Leu Ala Arg Glu Gly Ala Leu Val Tyr Cys Arg Glu Val Ash Lys Tyr Thr Ash Pro Arg Gly Gly Ash Phe G.n Pro Gly Leu Tyr

Pro Ser Ser Ile Asn Val Tyr Phe Gly Ser Leu Thr Gly Ala Thr Pro 645 650 655

Asp Gly Arg Lys Ser Gly Gln Pro Leu Ala Asp Gly Val Ser Pro Ser 660 665 670

Arg Gly Cys Asp Val Ser Gly Pro Thr Ala Ala Cys Asn Ser Val Ser 675 680 685

Lys Leu Asp His Phe Ile Ala Ser Asn Gly Thr Leu Phe Asn Gln Lys 690 695 700

Phe His Pro Ser Ala Leu Lys Gly Asp Asn Gly Leu Met Asn Leu Ser 705 710 715 720

Ser Leu Ile Arg Ser Tyr Phe Asp Gln Lys Gly Phe His Val Gln Phe 725 730 735

Asn Val Ile Asp Lys Lys Ile Leu Leu Ala Ala Gln Lys Asn Pro Glu 740 745 750

Lys Tyr Gln Asp Leu Ile Val Arg Val Ala Gly Tyr Ser Ala Gln Phe
755 760 765

Ile Ser Leu Asp Lys Ser Ile Glm Asn Asp Ile Ile Ala Arg Thr Glu
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His Val Met

785

<210> 7

<211> 304

<212> PRT

<213> Clostridium butyricum

<400> 7

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Ser Met Ser Cys Leu Trp Cys Ser Asn Pro Glu Ser Gln Asp Ile Lys
35 40 45

Pro Gln Val Met Phe Asn Lys Asn Leu Cys Thr Lys Cys Gly Arg Cys
50 55 60

Lys Ser Gln Cys Lys Ser Ala Gly Ile Asp Met Asn Ser Glu Tyr Arg 65 70 75 80

Ile Asp Lys Ser Lys Cys Thr Glu Cys Thr Lys Cys Val Asp Asn Cys
85 90 95

Leu Ser Gly Ala Leu Val Ile Glu Gly Arg Asn Tyr Ser Val Glu Asp

Val Ile Lys Glu Leu Lys Lys Asp Ser Val Gln Tyr Arg Arg Ser Asn 115 120 125

Gly Gly Ile Thr Leu Ser Gly Gly Glu Val Leu Leu Gln Pro Asp Phe 130 135 140 Ala Val Glu Leu Leu Lys Glu Cys Lys Ser Tyr Gly Trp His Thr Ala Ile Glu Thr Ala Met Tyr Val Asn Ser Glu Ser Val Lys Lys Val Ile Pro Tyr Ile Asp Leu Ala Met Ile Asp Ile Lys Ser Met Asn Asp Glu Ile His Arg Lys Phe Thr Gly Val Ser Ash Glu Ile Ile Leu Gln Ash Ile Lys Leu Ser Asp Glu Leu Ala Lys Glu Ile Ile Arg Ile Pro Val Ile Glu Gly Phe Asn Ala Asp Leu Gln Ser Ile Gly Ala Ile Ala Gln Phe Ser Lys Ser Leu Thr Asn Leu Lys Arg Ile Asp Leu Leu Pro Tyr His Asn Tyr Gly Glu Asn Lys Tyr Gln Ala Ile Gly Arg Glu Tyr Ser Leu Lys Glu Leu Lys Ser Pro Ser Lys Asp Lys Met Glu Arg Leu Lys Ala Leu Val Glu Ile Met Gly Ile Pro Cys Thr Ile Gly Ala Glu

<210> 8

<211> 385

<212> PRT

<213> Clostridium butyricum

<400> 8

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20 25 30

Lys Ala Leu Ile Val Thr Asp Lys Phe Leu Lys Asp Met Glu Gly Gly 35 40 45

Ala Val Glu Leu Thr Val Lys Tyr Leu Lys Glu Ala Gly Leu Asp Val
50 55 60

Val Tyr Tyr Asp Gly Val Glu Pro Asn Pro Lys Asp Val Asn Val Ile
65 70 75 80

Glu Gly Leu Lys Ile Phe Lys Glu Glu Asn Cys Asp Met Ile Val Thr
85 90 95

Val Gly Gly Ser Ser His Asp Cys Gly Lys Gly Ile Gly Ile Ala 100 105 110

Ala Thr His Glu Gly Asp Leu Tyr Asp Tyr Ala Gly Ile Glu Thr Leu 115 120 125

Val Asn Pro Leu Pro Pro Ile Val Ala Val Asn Thr Thr Ala Gly Thr
130 135 140

Ala Ser Glu Leu Thr Arg His Cys Val Leu Thr Asn Thr Lys Lys Lys
145 150 155 160

Ile Lys Phe Val Ile Val Ser Trp Arg Asn Lou Pro Leu Val Ser Ile 165 170 175

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Thr	Gly	Met	Asp	Ala	Leu	Thr	His	Ala	Ile	Glu	Ala	Tyr	Val	Ser	Lys
		195					200					205			
Asp	Ala	Asn	Pro	Val	Thr	Asp	Ala	Ser	Ala	Ile	Gln	Ala	Ile	Lys	Leu
	210					215					220				
Ile	Ser	Gln	Asr	Leu	Arg	Gln	Ala	Val	Ala	Leu	Glу	Glu	Asn	Leu	Glu
225					230					235					240
Ala	Arg	Glu	Asn	Met	Ala	Tyr	Ala	Ser		Leu	Ala	Gly	Met		Phe
				245					250					255	
													_		
Asn	Asn	Ala	Asn	Leu	Gly	Tyr	Val		Ala	Met	Ala	His		Leu	Gly
			260					265					270		
											• • •			v	D
Gly	Leu			Met	Ala	His		Val	FIA	Asn	EIA		∟eu	Lec	Pro
		275					280					285			
		1			Asn		1	Cox	7	Dro	I v o	Lvc	Dhe	7) a	ð en
als			Arg	lyr	ASII	295	Ti C. Ir	261	ASII	FIJ	300	Буз	-110	n_u	nop
	290					233					., 00				
Tlo	. או -	c Cla	. Dhe	. Ma∽	Gly	G n	Asn	Tie	Ser	Glv	Leu	Ser	Val	Met	Glu
305		GIC	r Fite	110	310		7.0			315					320
505	•				313					'					
Ala	. Al=	. Gli	Live	: Ala	lle	Asn	Ala	Met	Phe	Arq	Leu	Ser	Glu	Asp	Val
				325				. =	330					335	

Gly Ile Pro Lys Ser Lea Lys Glu Met Gly Val Dys Glu Asp Pho

340

345 350

. 6

Glu His Met Ala Glu Leu Ala Leu Leu Asp Gly Asn Ala Phe Ser Asn 355 360 365

Pro Arg Lys Gly Asn Ala Lys Asp Ile Ile Asn Ile Phe Lys Ala Ala 375

380 370

Tyr

385

<210> 9

<211> 35

<212> ADN

<213> Séquence artificielle

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<223 > Description de la séquence artificielle:Amorce

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40